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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification : Not classified	A2	(11) International Publication Number: **WO 95/02406** (43) International Publication Date: 26 January 1995 (26.01.95)

(21) International Application Number: PCT/US94/07240

(22) International Filing Date: 11 July 1994 (11.07.94)

(30) Priority Data:
08/092,045 15 July 1993 (15.07.93) US

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(81) Designated States: AU, CA, JP, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: BENZOTHIOPHENES AND BENZOFURANS AS AGENTS OF THERAPEUTIC USE IN THE TREATMENT OF INFLAMMATORY BOWEL DISEASE AND METHODS FOR ASSAYING INFLAMMATION

(57) Abstract

This instant invention is novel uses of known benzothiophenes or benzofurans and pharmaceutically acceptable salts thereof. Such compounds as 5-methoxy-3-(1-methylethoxy)-N-1H-tetrazol-5-ylbenzo[b]thiophene-2-carboxamide is used for treating inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. The instant invention is also an assay for noninvasively monitoring inflammation.

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BENZOTHIOPHENES AND BENZOFURANS AS AGENTS OF THERAPEUTIC USE IN THE TREATMENT OF INFLAMMATORY BOWEL DISEASE AND METHODS FOR ASSAYING INFLAMMATION

FIELD OF THE INVENTION

The present invention provides novel therapeutic uses of certain known benzothiophenes and benzofurans compounds. It has been found that these compounds are useful in the treatment of inflammatory bowel diseases such as Crohn's disease, ileitis, ischemic bowel disease, and ulcerative colitis. The present invention also provides the use of an assay which follows a biochemical marker that serves as a noninvasive index of inflammation.

BACKGROUND OF THE INVENTION

Inflammatory bowel diseases (IBD) are very common diseases in the Western world and with an increasing trend in developing countries. More than one million people suffer from them.

IBD can be broadly categorized into two different diseases, Crohn's disease and ulcerative colitis. Treatment regimens have many similarities even though the diseases have widely different presenting symptoms and their response to treatment varies. The introduction of sulfasalazine in the 1930's was a major advance in the treatment of IBD, although there was not widespread acceptance of its value until the mid-1950's. Until recently, treatment was limited to sulfasalazine and high-level immunosuppression with corticosteroids. Allergy and intolerance to

sulfasalazine and systemic side effects caused by corticosteroids have interfered with effective treatment. Over the past decade, new agents that have been utilized in IBD improve medical management by achieving a decrease in the medication-induced side effects and by increasing the number of patients entering and being maintained in remission.

There are multiple potential steps in the inflammatory response where specific agents can be utilized to modify the tissue damage that occurs in IBD. T and B lymphocytes, polymorphonuclear leukocytes, mast cells, eosinophils, basophils, tissue macrophages, and monocytes all have a vital role in the cellular inflammatory response. The inflammatory response is an essential reaction to environmental stimuli, but, in the process of protection, many toxic compounds such as eosinophilic basic protein and oxygen-derived free radicals invoke damage to the gastrointestinal mucosa. Modifying the inflammatory response may be useful in limiting the tissue damage while not interfering with the beneficial aspect of inflammation.

Many cells participate in the inflammatory process through a number of different molecular mechanisms. An important role is played by the arachidonic acid pathway, which gives rise to the leukotrienes and prostaglandins. Treatment of IBD has been targeted toward specific areas in this complex system to modify the immune response. Agents such as corticosteroids and muzolimine (5-aminosalicylic acid, 5-ASA) influence several sites in the inflammatory response. Other agents work in only one site. Current research is investigating which of these sites are the most significant in the inflammatory response in IBD.

Despite all of the advances represented by the use of the foregoing compounds, effective treatment of IBD

is still the goal of many researchers. Nonspecific treatment of IBD with these compounds continues to be associated with problems. One such problem is that these compounds are not effective in treating intolerant or allergic patients.

Recently, an animal model of experimental colitis, induced by intracolonic administration of trinitrobenzens sulfonic acid (TNBS) dissolved in ethanol, was developed. This model resembles human IBD, particularly Crohn's disease, in both histological and morphological features. For instance, the inflammation is transmural and includes granulomas. Morphologically, skip-segment ulceration and inflammation are common, and the mucosa frequently has a "cobblestone"-like appearance. The model is useful for the study of the etiopathogenesis in chronic colonic inflammation and events characterizing the progression from acute to chronic inflammation. Morris, et al., Gastroenterology 1989;96:795-803.

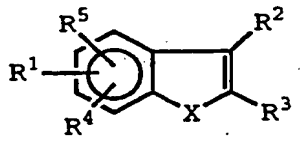
This TNBS model of IBD has been used by many researchers in the field and has been described in numerous papers. Wallace and Keenan (Am J Physiol 1990;258:G527-G534) examined an orally active inhibitor of leukotriene synthesis referred to as MK-886; Andersen, et al., Scand J Castroenterol 1992;27:757-763, examined the water-soluble contrast medium iodixanol; Hoshino, et al., Clinical and Experimental Pharmacology and Physiology 1992;19:717-722, examined various prostaglandins; Kim, et al., Scand J Gastroenterol 1992;27:529-537, reviewed various compounds which induced colitis in various animal models; Yamada, et al., Gastroenterology 1992;102:1524-1534, also examined various compounds which induced colitis in various animal models; and Wallace, et al., Inflammation 1992;16;4:343-354, pretreated the colonic epithelium with a monoclonal

antibody (IB-4) directed against the leukocyte adhesion molecule, CD18, and markedly suppressed neutrophil infiltration into the colonic tissue after induction of colitis. There is no disclosure in these references to suggest the compounds, formulations, or methods of using the formulations of the present invention to treat IBD.

United States Patent 4,703,053 issued October 27, 1987 to Connor, et al., cover the compounds of the instant invention, methods for preparing them, and a use thereof. The use disclosed was for antiallergic purposes. This patent is hereby incorporated by reference. There is no disclosure in this patent to suggest the compounds, formulations, or methods of using the formulations of the present invention to treat IBD.

SUMMARY OF THE INVENTION

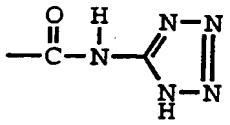
The present invention is concerned with the use of certain known benzothiophenes and benzofurans of the formula



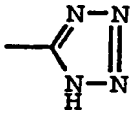
I

wherein (1) R^1, R^4, and R^5 are independently H, alkyl of from 1 to 12 carbons, inclusive, alkoxy of from 1 to 12 carbons, inclusive, hydroxy, aryl, R^1 taken twice having each on adjacent carbons such that the two R^1s together are methylenedioxy, nitro, amino, substituted amino, mercapto, alkylthio of from 1 to 4 carbons

inclusive, alkylsulfinyl of from 1 to 4 carbons, inclusive, alkylsulfonyl of from 1 to 4 carbons, inclusive, arylthio, arylsulfinyl, arylsulfonyl, or halogen; (2) R^2 is H, alkyl of from 1 to 12 carbons, inclusive, alkoxy of from 1 to 12 carbons, inclusive, arylmethoxy, amino, substituted amino, mercapto, alkylthio of from 1 to 4 carbons, inclusive, alkylsulfinyl of from 1 to 4 carbons, inclusive, alkylsulfonyl of from 1 to 4 carbons, inclusive, arylthio, arylsulfinyl, or arylsulfonyl, and (3) R^3 is A or B



A



B

as well as of the pharmacologically acceptable salts of Formula I for the preparation of pharmaceutical compositions for use in the treatment of IBD.

The present invention also provides an assay which follows inflammation in vitro or in vivo, regardless of its etiology. The assay follows a biochemical marker that serves as a noninvasive index of inflammation. Preferably, urine is collected from an individual, and the urine is analyzed for the presence of the biochemical marker, 8-hydroxydeoxyguanosine, as an indicator of inflammation.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is described by way of example with reference to the accompanying drawings.

Figure 1 shows how trinitrobenzenesulfonic acid (TNBS) exposure increased the average rate of 8-OH-dGUA excretion in a rat model by 276% over controls. 5-methoxy-3-(1-methylethoxy)-N-1H-tetrazol-5-ylbenzo[b]thiophene-2-carboxamide monosodium salt reduced this increase to levels statistically indistinguishable from controls.

Figure 2, similar to Figure 1 and a repetition of the first experiment, shows how exposure to trinitrobenzenesulfonic acid (TNBS) increased the average rate of 8-OH-dGUA excretion in a rat model by 349% and 254% over controls. 5-Methoxy-3-(1-methyl-ethoxy)-N-1H-tetrazol-5-ylbenzo[b]thiophene-2-carboxamide monosodium salt reduced this increase to levels statistically indistinguishable from controls.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Several terms used herein are defined as follows:

Alkyl of from 1 to 4 carbons, inclusive, is methyl, ethyl, propyl, butyl, or isomeric forms thereof.

Alkyl of from 1 to 12 carbons, inclusive is methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl octyl, nonyl, etc, and includes isomeric forms of the alkyl of from 1 to 6 carbons, inclusive. Alkyl of from 1 to 6 carbons, inclusive, are preferred.

Alkoxy of from 1 to 12 carbons, inclusive, is methoxy, ethoxy, propoxy, butoxy, etc, and includes isomeric forms of alkoxy of from 1 to 6 carbons, inclusive. Alkoxy of from 1 to 6 carbons, inclusive are preferred.

Substituted amino in mono- or di-alkylamino wherein the alkyl may be the same or different from 1 to 6 carbons when taken alone or together.

Halogen is chloro, bromo, fluoro, iodo, or trifluoromethyl.

Aryl is phenyl or substituted phenyl having 1 or 2 substitutions, such as halogen, alkyl of from 1 to 6 carbons, inclusive, alkoxy of from 1 to 6 carbons, inclusive, hydroxy, nitro, amino, substituted amino, and the like.

Processes for the preparation of compounds of general Formula I have already been described in US Patent 4,703,053 issued October 27, 1987, to Connor, et al. As indicated above, this patent is incorporated herein by reference.

Pharmaceutical compositions are prepared from compounds of Formula I and salts thereof described as the present invention having inert pharmaceutical carriers. Inert, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, dispersible granules, capsules, cachets, and suppositories. A solid carrier can be 1 or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material. In

powders, the carrier is a finely divided solid which is in admixture with the finely divided active compound of Formula I. In the tablet the active compound is mixed with carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain from 5% or 10% to about 70% of the active ingredient. Suitable solid carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low melting wax, cocoa butter, and the like. The term "preparation" is intended to include the formulation of the active compound with encapsulating material as carrier providing a capsule in which the active component (with or without other carriers) is surrounded by carrier, which is thus in association with it. Similarly, cachets are included. Tablets, powders, cachets, transdermal and transmucosal systems, and capsules can be used as solid dosage forms suitable for oral administration.

Liquid form preparations include solutions, suspensions, and emulsions. As an example, water or water-propylene glycol solutions may be mentioned for parenteral injection. Liquid preparations can also be formulated in solution in aqueous polyethylene glycol solution. Aqueous solutions suitable for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizing, and thickening agents as desired. Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, i.e., natural or synthetic gums, resins, methyl cellulose, sodium carboxymethyl cellulose, and other well-known suspending agents. Preferably, the pharmaceutical preparation is in unit dosage form. In

such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, for example, packeted tablets, capsules, and powders in vials or ampoules. The unit dosage form can also be a capsule, cachet, or tablet itself or it can be the appropriate number of any of these in packaged form.

The compounds of structural Formula I can be prepared and administered in a wide variety of oral and parenteral dosage forms. The compounds of structural Formula I compounds can also be administered intravenously. For example, a useful oral dosage is between 1 and 50 mg/kg, a useful parenteral dosage is between 1 and 50 mg/kg, and a useful intravenous dosage is between 1 and 50 mg/kg.

The quantity of active compound in a unit dose of preparation may be varied or adjusted from 1 to 100 mg according to the particular application and the potency of the active ingredient.

In therapeutic use as agents for treating IBD, the compounds utilized in the pharmaceutical method of this invention are administered at the initial dosage of about 0.1 mg to about 21 mg/kg daily. A daily-dose range of about 0.35 mg to about 12 mg/kg is preferred. The dosages, however, may be varied depending upon the requirements of the patient, the severity of the condition being treated and the compound being employed. Determination of the proper dosage for a particular situation is within the skill of the art. Generally, the treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total

daily dosage may be divided and administered in portions during the day if desired.

As indicated above, the present invention also provides an assay which follows inflammation in vitro or in vivo, regardless of its etiology. The assay follows a biochemical marker that serves as a noninvasive index of inflammation. The assay is surprisingly advantageous because of its noninvasive character.

Inflammation is usually monitored in animal models by sacrificing the animal and assessing the histopathology at the site of infusion of inflammatory mediators, a process which requires looking at different animals at each time point. To circumvent this limitation, the present invention employs an HPLC assay to follow a biochemical urinary marker that serves as a noninvasive index of inflammation. Regardless of etiology, free radicals figure prominently among the proximate mediators of all inflammation, so that the quantification of some product of free radical reactions can therefore serve as an indicator of inflammation. DNA is susceptible to hydroxylation by free radicals produced during inflammation. If they become hydroxylated, DNA bases are enzymatically excised and subsequently excreted intact into the urine. For example, deoxyguanosine is preferentially hydroxylated at the 8' site by hydroxyl radical attack to form 8-hydroxydeoxyguanosine, which subsequently appears intact in the urine where it can be quantified.

The evidence provided herein that the benzothiophene and benzofuran compounds of the present invention moderate inflammatory bowel disease (IBD) is based on examining the urinary excretion of 8-hydroxy-deoxyguanosine (8-OH-dGUA) as an indicator of in vivo hydroxyl radical production.

The urinary excretion is preferably analyzed by high-pressure liquid chromatographic procedures. Nevertheless, it should be understood that the present invention is not limited as such. Those of ordinary skill in the art will know different analytical tools for detecting a biochemical marker from a sample.

In the preferred embodiment, the urine sample is partially purified using an ion exchange solid phase extraction resin. These resins are known to those of skill in the art. The partially purified sample is then run out on high-pressure liquid chromatography, preferably using an electrochemical detector to qualify the biochemical marker peak.

The noninvasive use of this assay to follow inflammation regardless of its etiology is beneficial; a urine test will be a welcome alternative in the clinic where many inflammatory responses are followed invasively. For example, a urine collection to monitor the progress of IBD over time will be clearly preferable to the current practice of repeated endoscopic examinations. Moreover, an assay such as the one described herein which is a noninvasive index of radical production will serve to monitor any other diseases where radicals play a role, and such a list includes a host of ailments.

The method of using this invention is further elaborated by the representative examples as follows.

EXAMPLE 1

As previously described, a rat model of IBD was used in this example where trinitrobenzenesulfonic acid (TNBS) was instilled into the bowel at the start of the experiment to initiate an inflammatory response. Ethanol was used to dissolve the TNBS and to breach the mucous barrier. TNBS-induced inflammation occurred in

a dose-dependent manner, and persisted for at least several weeks before moderating. Administration of benzothiophene and benzofuran compounds such as 5-methoxy-3-(1-methylethoxy)-N-1H-tetrazol-5-ylbenzo[b]thiophene-2-carboxamide to the rat model in this example demonstrated substantial improvement to bowel lesions in the rat.

At the start of the experiment, groups of eight male Wistar rats were given under light ether anesthesia an enema of saline in 50% ethanol (controls) or trinitrobenzenesulfonic acid (TNBS; 75 mg/kg) in 50% ethanol. Animals thereafter received via oral gavage either saline or 7.5 mg/kg of the test compound, 5-methoxy-3-(1-methylethoxy)-N-1H-tetrazol-5-ylbenzo[b]thiophene-2-carboxamide, dissolved in saline on a daily basis. Urine was collected overnight from animals housed individually in metabolic cages, the volume was measured, and an aliquot frozen. The urine was partially purified using an ion exchange solid phase extraction resin, and the sample was run out on HPLC using an electrochemical detector to quantify the 8-OH-dGUA peak. Standards were verified using mass spec and fast atom bombardment techniques.

The experiment was repeated twice. In Experiment 1, excretion of 8-OH-dGUA was followed in 3 groups of 8 animals each (N = 24) for 15 days after the initial ethanol or ethanol-TNBS exposure. Experiment 2 was identical to Experiment 1 except that the experiment lasted 20 days.

RESULTS

In Experiment 1, TNBS exposure increased the average rate of 8-OH-dGUA excretion by 276% over controls (Figure 1). The test compound reduced this

increase to levels statistically indistinguishable from controls. The average excretion rate presented for each group was the average of 104 observations (eight animals for 13 nights over a 15-day period), for an overall total of 312 observations. Means not significantly different at p >0.001 share superscripts (ANOVA, F = 8.76, Bonferroni multiple comparison).

Experiment 2 contained two groups exposed to TNBS and not given the test compound; TNBS exposure increased the average rate of 8-OH-dGUA excretion by 349% and 254% over controls (Figure 2). Daily oral dosage of the test compound reduced this TNBS-induced increase to levels statistically indistinguishable from controls. The average excretion rate presented for each group was the average of 160 observations (8 animals for 20 nights), for an overall total of 637 observations. Means not significantly different at p >0.001 share superscripts (ANOVA, F = 19.27, Bonferroni multiple comparison).

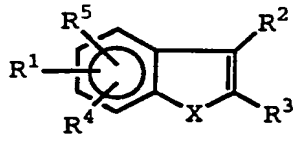
No individual animal in any of the treatments was significantly different from its peers, i.e., no single animal inordinately skewed the average for its group.

The present invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within their scope.

What is claimed is:

CLAIMS

1. A method for treating inflammatory bowel disease which comprises administering a therapeutically effective amount of a compound of formula



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wherein (1) R^1, R^4, and R^5 are independently H, alkyl of from 1 to 12 carbons, inclusive, alkoxy of from 1 to 12 carbons, inclusive, hydroxy, aryl, R^1 taken twice having each on adjacent carbons such that the two R^1s together are methylenedioxy, nitro, amino, substituted amino, mercapto, alkylthio of from 1 to 4 carbons inclusive, alkylsulfinyl of from 1 to 4 carbons, inclusive, alkylsulfonyl of from 1 to 4 carbons, inclusive, arylthio, arylsulfinyl, arylsulfonyl, or halogen; (2) R^2 is H, alkyl of from 1 to 12 carbons, inclusive, alkoxy of from 1 to 12 carbons, inclusive, arylmethoxy, amino, substituted amino, mercapto, alkylthio of from 1 to 4 carbons, inclusive, alkylsulfinyl of from 1 to 4 carbons, inclusive, alkylsulfonyl of from 1 to 4 carbons, inclusive, arylthio, arylsulfinyl, or arylsulfonyl, and (3) R^3 is A or B

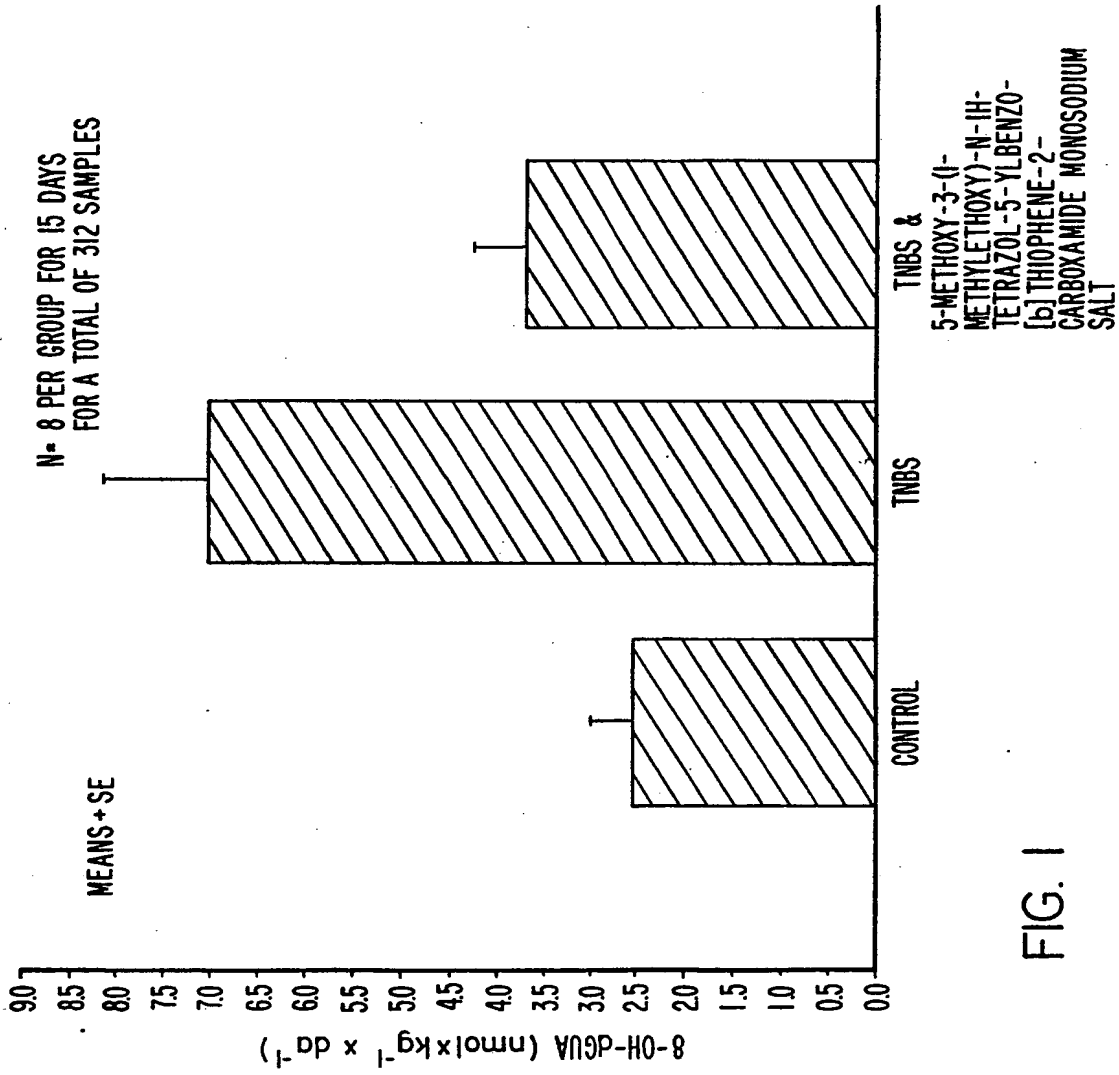
8-OH-dGUA (nmol×kg⁻¹ × da⁻¹)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
MEANS+SE
N= 8 PER GROUP FOR 15 DAYS
FOR A TOTAL OF 312 SAMPLES
CONTROL
TNBS
TNBS &
5-METHOXY-3-(1-METHYLETHOXY)-N-1H-TETRAZOL-5-YLBENZO-(b)THIOPHENE-2-CARBOXAMIDE MONOSODIUM SALT

FIG. 1

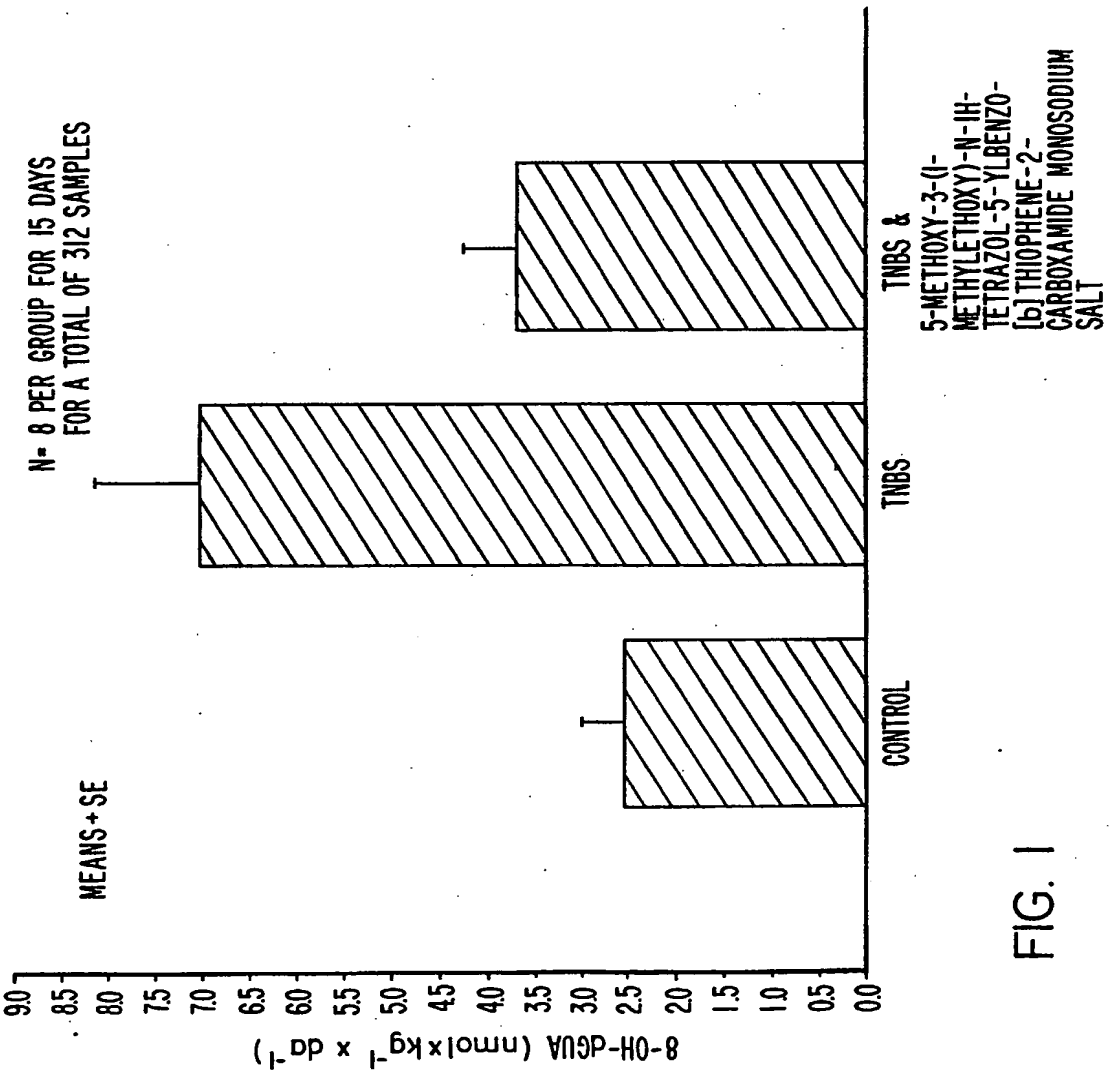
8-OH-dGUA (nmol×kg⁻¹ x da⁻¹)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
MEANS+SE
N= 8 PER GROUP FOR 15 DAYS
FOR A TOTAL OF 312 SAMPLES
CONTROL
TNBS
TNBS &
5-METHOXY-3-(1-METHYLETHOXY)-N-1H-TETRAZOL-5-YLBENZO-[b]THIOPHENE-2-CARBOXAMIDE MONOSODIUM SALT

FIG. 1

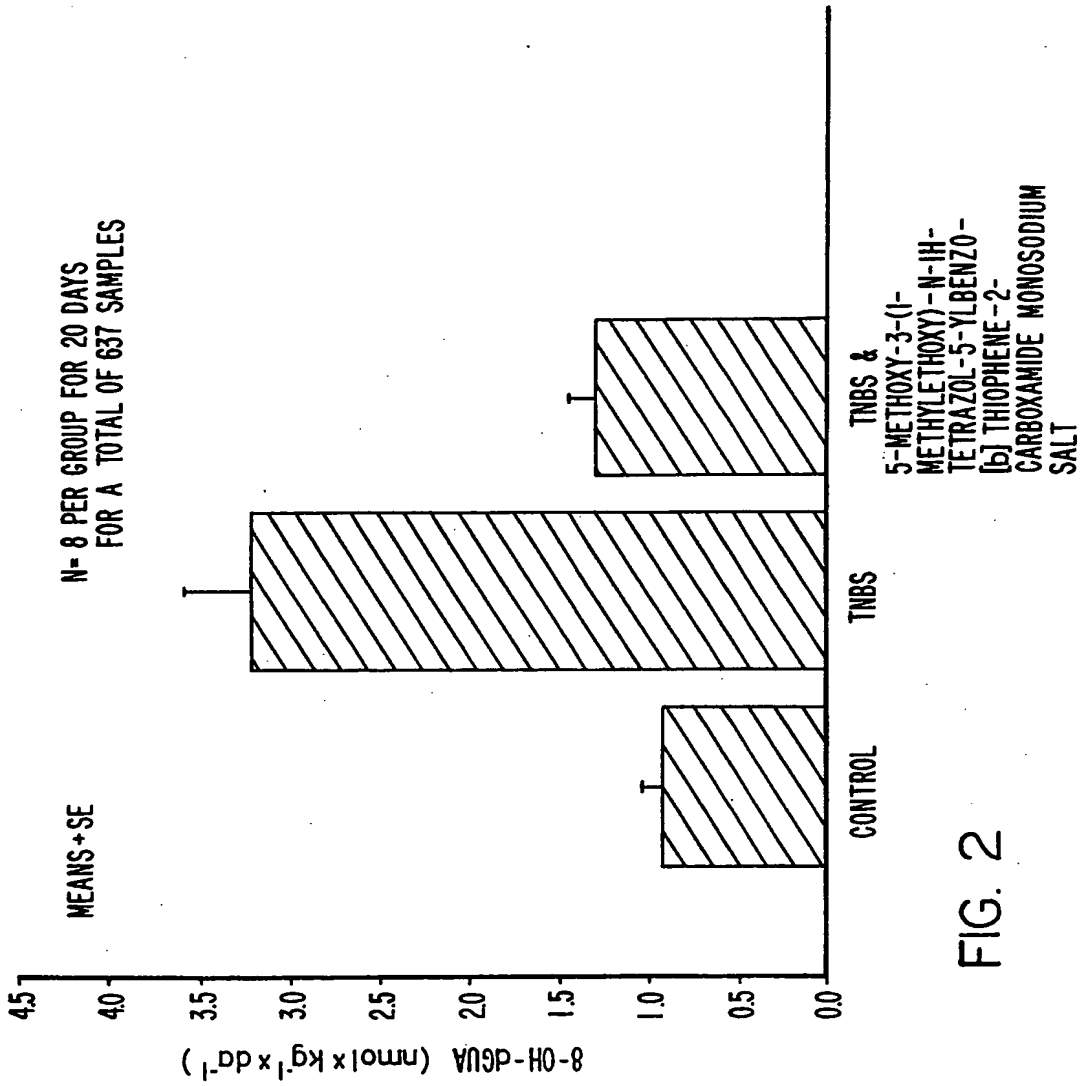
8-OH-dGUA (nmol x kg-1 x da-1)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
MEANS+SE
N= 8 PER GROUP FOR 20 DAYS
FOR A TOTAL OF 637 SAMPLES
CONTROL
TNBS
TNBS &
5-METHOXY-3-(1-
METHYLETHOXY)-N-1H-
TETRAZOL-5-YLBENZO-
(b) THIOPHENE-2-
CARBOXAMIDE MONOSODIUM
SALT

FIG. 2